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CONTENTS.

THE ANATOMY AND HISTOLOGY OF THE ADULT FEMALE MOSQUITO.

By S. R CHRISTOPHERS, M.B. Vict.

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[Plates 1—6.]

The structure of the Mosquito has become of considerable importance since the discovery by Ross of the changes undergone by Proteosoma in a species of mosquito. Moreover, a knowledge of the structure of mosquitoes is necessary in following out not only the development of the parasites of bird and human malaria, but also of Filaria, and possibly of other disease-producing parasites.

We have therefore in the present article described more fully than has yet been done not only the gross anatomy, but also the minute structure, of the organs and tissues of Culex and Anopheles.

The Culicidæ are a highly-specialised group of Diptera. Their muscular, respiratory, vascular, and reproductive systems are to a large extent similar to those of other Diptera.

The genera of Culicidæ do not differ very much from one another in their general anatomy, and still less in the minute structure of their tissues. The differences between Culex and Anopheles, apart from external characters, are chiefly to be found in a generally more robust exoskeleton in Culex, and in the possession of sacculated salivary glands by Anopheles, whereas those of Culex are strictly tubular.

Part I.—The Gross Anatomy.

The Exoskeleton.

As in most insects, the body consists of head, thorax, and abdomen. (Plate 1, fig. 1.)

The Head.—A large portion of the head is formed by the large compound eyes. These occupy the whole of the lateral portions of the head, and approach very close to one another anteriorly. Inferiorly they actually meet in the middle line. In the space between the eyes are the large basal joints of the antennæ. Beneath the origin of the antennæ are the combined clypeus and labrum with the proboscis.

The head is connected with the thorax by a narrow membranous neck, in which are two lateral chitinous plates (cervical sclerites).

The Thorax.—This, as in other insects, consists of three segments, the pro-, meso-, and meta-thorax. Of these, as in other diptera, the mesothorax is the larger, and the prothorax is very small.

Each segment consists of a dorsal piece or notum, a ventral piece or sternum, and a lateral portion or pleuron. In the well-developed mesothorax the notum consists of several portions, of which the large scutum and the smaller scutellum and post-scutellum are readily seen. In the other segments these divisions are not readily made out. Both in the meso- and meta-thorax the pleuron consists of two large plates, the epi-merum and epi-sternum of each segment respectively.

The prothorax is collar-like in shape. The pronotum is undeveloped, but on either side of the base of the neck are two conspicuous processes, which consist of two freely movable plates (patagia).

From these, on either side, pass downwards the two rod-shaped pleural bodies connected below with the prosternum.

The mesothorax forms the greater part of the thorax. There is a large ovoid scutum. Posterior to the scutum there is a thick transverse ridge, the scutellum, which shows very prominently in longitudinal sections. Posterior to this, and forming the roof of the thorax behind the wings, is a large plate, which sends inwards a process, and to which the posterior portion of the great antero-posterior wing-muscle group is attached. The mesosternum forms two large surfaces behind the first pair of legs, and projects laterally above the middle coxa. The episternum and epimerum are two large plates placed laterally. In the pleuron of the mesothorax is the largest spiracle of the body, the first thoracic stigma. Several small detached plates are present in its neighbourhood.

The metathorax is narrow and ring-like. It bears the halteres or homologues of the second pair of wings in neuroptera, &c., and also a large spiracle, the second thoracic stigma. From the notum and sternum large chitinous processes project inwards (apodemes). These give attachment to both thoracic and abdominal muscles.

The Abdomen.—The abdomen consists of eight segments. Each consists of a tergum and sternum connected laterally by the pleural membrane. The pleural membrane continues unbroken throughout the length of the abdomen, and carries the abdominal spiracles, one opposite each segment. From the last segment project two flap-like processes, which are used in the deposition of ova.

The Wings.—The wings arise from the mesothorax. They are not directly associated with the main masses of wing muscles which are inserted into the walls of the thorax. The variation in the shape of the thorax caused by the contractions of the muscles causes the up and down movement of the wings.

The Legs.—The legs arise from each segment of the thorax. The proximal joint is the large coxa. Between this and the femur is the small trochanter. The other joints are the tibiæ and tarsi.

The Alimentary Canal.

The alimentary canal is specialised on account of the blood-sucking habits of the mosquito. It differs from many insects in not possessing any cæcal diverticula of the mid-gut. It also differs in the possession of five Malpighian tubules, these being in insects usually even in number. (Plate 1, fig. 2.)

The parts of the alimentary canal are as follows :—

- The mouth
- The pharynx with pumping organ
- The œsophagus
- The œsophageal diverticula

} The fore-gut.

- The homologue of the proventriculus
- The stomach (so-called)
- The pylorus

} The mid-gut.

- The pyloric dilatation
- The small intestine
- The colon
- The rectum with rectal papillæ

} The hind-gut.

The mouth, pharynx, and œsophagus are ectodermal in origin, and both the mouth and pharynx are lined with chitin. The hind-gut is also ectodermal in origin ; it does not possess, however, any portion lined with chitin. The mid-gut is the true digestive portion of the tract.

The Pharynx.—The pharynx, which is lined throughout its extent with chitin, passes upwards and backwards through the ganglionic ring formed by the supra- and infra-œsophageal ganglia and their commissures. At first it is narrow, but posteriorly becomes a large chamber (the pumping organ).

The pumping organ occupies with its muscles a large portion of the head behind the level of the cerebral ganglia. In the state of rest its lumen is triradiate in transverse section. The walls are formed of three large and thick chitinous plates, one placed on either side and one superiorly. Into each of these plates powerful muscles are inserted. Those of the superior plate consist of two muscular masses, taking their origin from the occiput. Those of the lateral plates consist on each side of a single large muscular mass arising from the lateral portions of the head. The plates are connected by thin non-chitinous membrane, and their edges are rolled so that they form a

spring capable of returning to their original position so soon as the separating force of the muscles ceases. (Plate 1, figs. 3 and 4.)

Posteriorly, where the pharynx becomes very narrow, a sharp bend occurs and a valvular action is produced. The whole forms a very powerful suctorial apparatus.

The Œsophagus.—Immediately beyond the pumping organ the chitinous layer ceases, and the rest of the fore-gut is formed of excessively thin membrane. At the junction of the two portions a sharp bend occurs, and the floor projects so as to form a valvular flap.

The thin-walled œsophagus is a large dilated sac, whose walls are supported by surrounding structures. Into the posterior wall of the dilated and thin-walled œsophagus projects the papilla-like anterior portion of the mid-gut.

The Diverticula of the Œsophagus.—From the œsophagus two or three diverticula, similar in nature to the œsophagus, extend backwards. Of these one is of great size, and usually contains air. This most usually extends into the abdomen, and is a prominent object in dissections and sections. In the newly-hatched mosquito it is small, but rapidly becomes large enough to extend into the abdomen. (Plate 1, fig. 3.)

The Homologue of the Proventriculus.—There is no true proventriculus as in many insects. There is, however, an interesting fold of the fore-gut into the mid-gut which represents this organ. The anterior portion of the mid-gut has been noted as projecting into the dilated œsophageal pouch. This portion consists of both ectodermal and endodermal portions, and represents the proventriculus in other insects (see "Histology," Part II). The muscular bundles are here increased, and the whole forms a valvular muscular organ. (Plate 1, fig. 3.)

The Mechanism of Feeding.—The powerful pumping action which must result from a drawing asunder of the three large chitinous plates of the pumping organ is very evident. These plates, also, when drawn apart must, by reason of their spring-like shape, revert to their original positions close together, without any muscular aid. Posteriorly the valve-like arrangement mentioned before prevents regurgitation.

In mosquitoes as usually killed, the proventriculus and anterior portion of the mid-gut are considerably distant from the posterior end of the pumping organ, so that the large delicate walled œsophageal chamber with its extensive diverticula intervene. Immediately after feeding, however, though blood is very evident in the mid-gut, and even in the calyx-like proventriculus, yet in the œsophagus there is no trace. As this latter is so large and has such delicate walls, it is evident that, in the act of feeding, the calyx-like proventriculus must be applied directly to the posterior opening of the pharynx, thus shutting off the capacious œsophageal pouch. The large œsophageal

diverticulum probably acts, not only as an air chamber to specifically lighten the body of the mosquito, but also as an air pad to distribute the pressure of the large coagulum formed in the mid-gut after feeding. In a fed mosquito a transparent area is generally to be seen in front of the opaque mass of blood in the abdomen. This transparent area is the abdominal portion of the air-containing œsophageal diverticulum. (Plate 1, fig. 3.)

The Mid-gut.—The mid-gut extends from the proventriculus to the origin of the Malpighian tubes. It consists of two portions which merge into one another—an anterior narrow portion, and a large dilated posterior portion, which becomes greatly distended after feeding. Unlike most insects there are no cæcal appendages in the mosquito. Posteriorly there is a marked constriction, with strong muscular bundles, which forms a very marked pylorus. (Plate 1, fig. 2.)

The anterior narrow portion of the mid-gut lies in the thorax, and does not become distended with blood. The posterior portion when fully dilated fills the greater portion of the abdomen, the viscera being pushed into the last few segments. (Plate 3, fig. 2.)

The Hind-gut.—The hind-gut is short and passes in one or two bends from the pylorus to the anus. Immediately beyond the pylorus there is a considerable dilatation which is poorly supplied with muscular fibres : into this open the five Malpighian tubules. For a short distance beyond this the lumen is narrow (small intestine), but becomes gradually larger (colon). At the termmation of the colon there is a slight constriction, after which the canal dilates again to form the rectum. (Plate 1, fig. 2 ; also Plate 3, fig. 1.)

Into the rectum project six solid growths, the so-called rectal glands, which are, however, papillæ. Posteriorly the rectum ends in the anus close above the gynæphoric canal.

The appendages of the alimentary canal are :—

The Salivary Glands.—The salivary glands consist of six tubular acini lying three upon either side. Those of one side lie generally one above the other in the long axis of the body, their anterior ends lying close against the prosternum, where the ducts coming from each acinus unite to form a single duct. The upper and middle acini generally lie with their distal ends close to the proventriculus. The lower acinus passes towards the thoracic ganglion. Occasionally an acinus becomes bifid at a short distance from its termination. A common abnormality also is a small accessory acinus near the proximal end of an acinus. A duct can be seen traversing almost the entire length of each acinus. Shortly after leaving the acinus the three unite to form a single duct. The duct of each side passes up into the neck, and lies close to the nerve cords passing between the thoracic and the cerebral ganglia. Beneath, and in contact with the lower surface of the sub-œsophageal ganglion, the ducts of each side unite to form a common

salivary duct which passes forwards and enters the chitinous first portion of the alimentary canal close to the base of the proboscis.

The Malpighian Tubules.—These are five in number and open into the first portion of the hind-gut immediately beyond the pylorus. Their blind ends are held in position in the neighbourhood of the rectum by tracheal branches. They pass forwards in loops above their origin, so that, in transverse section, as many as ten may be seen cut across.

The Muscular System.

The chief muscular masses in the mosquito are contained in the thorax. They are chiefly muscles moving the wings and legs.

Wing Muscles.—There are two large muscular masses on either side of the thorax, passing from the dorsal to the ventral body wall. Between these bundles there is a space, in the lower portion of which lies the alimentary canal, main air tubes, and other structures. The upper portion of the space is occupied by a second series of large muscular bundles, passing from the front to the back of the thorax. Neither of these large masses of muscle are inserted directly into the wings, the up and down movement of the wings being caused by alterations in the shape of the thorax, consequent on the contractions of the vertical and horizontal fibres respectively. (Plate 2, figs. 4 and 5.)

There are, however, a few fibres arising from the lateral portions of the thorax, and inserted about the base of the wings.

Leg Muscles.—These occupy but little space in the thorax. They rise to a large extent from the internal processes of the exoskeleton (apodemes), and are inserted into neighbouring portions of the limbs. They arise also from one segment of a limb and are inserted into another.

The Muscles of the Body Segments.—These arise from one segment and are inserted into the next. They are arranged dorsally and ventrally in lateral groups throughout the abdomen.

A small muscle is also situated on each side, passing vertically from the tergum to the sternum. These on contracting flatten the abdomen.

Muscles in association with the Alimentary Canal.—Several important muscular masses are connected with the large chitinous pumping organ. A pair of muscles arises from the occipital region of the exoskeleton, and is inserted into the upper plate of the organ. A large muscle arises on each side, and is inserted into each of the lateral plates.

In the thorax a small muscular band rises from the neighbourhood of the first pair of legs, and passes upwards close to and outside the salivary glands of each side. The contraction of this band must exert pressure upon the salivary glands. (Plate 2, figs. 1 and 2.)

Anteriorly and posteriorly small muscular bundles pass from the dilated portion of the mid-gut to the abdominal wall.

The Tracheal System.

Respiration is entirely carried on by tracheæ. These take their origin from external openings—the spiracles, and eventually terminate in minute capillaries in the actual tissues of the insect. In culex and anopheles there is no development of large or multiple air sacs in connection with the tracheal system, as in many insects. In their case probably the large œsophageal diverticulum plays the same part.

The spiracles are placed both in the thorax and in the abdomen. The thoracic spiracles are two in number, situated in the meso-thoracic and meta-thoracic segment respectively. Of these the anterior one is the largest in the body. The second thoracic spiracle is also much larger than the abdominal spiracles. The abdominal spiracles are situated in the pleural membrane, one in each segment. (Plate 1, fig. 1.)

The Tracheæ.—Very large tracheæ pass inwards from the anterior thoracic spiracles. (Plate 2, fig. 3.)

1. A large branch passes forwards towards the neck and gives off a branch which passes down on either side of the middle line to the two anterior coxæ and the salivary glands. The main branch continues on through the neck, and supplies the head with numerous large branches.

2. A large branch passes upwards and backwards along the edge of the meso-scutum, and gives off branches which supply the wing muscles. A smaller branch also passes forwards and supplies the muscles of the thorax.

3. The largest trachea in the body (main trachea) passes downwards, backwards, and inwards, so as to lie on either side of the anterior portion of the alimentary canal. Numerous branches are given off from this trunk to the thoracic muscles, the alimentary canal, and legs. Posteriorly the trunk is continuous with a trachea passing forwards from the second thoracic spiracle, thus forming on either side a large tracheal loop.

Large tracheæ also pass inwards from the posterior thoracic spiracles.

1. Branches pass forwards and join in a loop with the main trachea, also backwards to join the abdominal system.

2. Branches pass downwards to the meta-thorax and posterior pair of legs.

3. Branches pass inwards to the muscles and mid-gut.

From each abdominal spiracle a short thick trunk passes inwards which gives rise to the following branches:—

A dorsal branch ramifying beneath the tergum and joining the branch of the opposite side.

A sternal branch supplying the sternal plate and muscles, also joining the branch of the other side.

Loop branches passing to the trunks anterior and posterior.

Branches passing inwards and supplying viscera. Branches from the first, second, third, and fourth abdominal tracheæ supply mainly the mid-gut, those from the fourth and fifth the ovaries, those from the sixth and seventh the genital organs.

The Vascular System.—As in most insects where the respiratory system ramifies throughout the whole body, the vascular system is not well developed. A dorsal vessel or heart and an anterior prolongation of this (aorta) are the only closed blood-vessels. Apart from the dorsal vessel the blood circulates in large blood spaces, which lie between the lobes of the fat-body and among the muscles and viscera.

The dorsal vessel passes close beneath the tergal plates throughout the abdomen. It is very thin walled, and is not provided with valves. The upper portion is attached to the dorsum at intervals by suspensory fibres (muscular), so that a festooned appearance is given in longitudinal section. There is, however, no true division into compartments. Laterally large cells (pericardial cells) are arranged throughout its entire extent, and fibres of a muscular nature (alary muscle) pass from the body wall and end in branches in close connection with the dorsal vessel (see "Histology," Part II). (Plate 4, fig. 1.)

At the first abdominal segment the dorsal vessel dips down beneath the mesophragma, lying as it does so, in direct contact with the cuticle. In the thorax it again arches upwards, and lies between the lower portions of the antero-posterior wing muscles close above the anterior portion of the mid-gut.

In the anterior third of the thorax it divides into two smaller portions which pass outwards, and coming in contact with the salivary ducts enter the neck.

Blood spaces without definite walls occur throughout the body. The thorax especially contains large spaces among the muscles, and the complex fat-body which lies between and supports the organ is everywhere bathed with blood fluid. (Plate 1, fig. 3.)

The Nervous System.

The ganglionic system in the Culicidæ is considerably developed. The head ganglia are large and complex. The thoracic ganglia are large and compressed so as to form a large ganglionic mass. The ganglia of this system are as follows:—

a. Lying around the pharynx is a ganglionic ring composed of large supra- and infra-œsophageal ganglia with their commissures. From these, large nerves go to the eyes, antennæ, and mouth parts.

b. In the thorax lying below the œsophageal diverticulum and close to the sterna is a large compound ganglion showing evidence of its

origin from the conjoined ganglia. Between this and the head ganglia are two long slender nerve cords, which pass in the neck in close relation with the salivary ducts. From the thoracic ganglion large nerves pass to the limbs, and posteriorly nerve cords connect it with the first abdominal ganglion.

c. The abdominal ganglia lie with their connecting commissures close upon the abdominal sterna. The last ganglion lies just below the junction of the oviducts to form the common oviduct. A large nerve passes from it among the viscera of the last few segments.

The Visceral System.—Small ganglia connected with the main ganglionic system occur in connection with the viscera. The most important of these are two small groups of large nerve cells lying in front of and above the thoracic ganglion, with the middle portion of which they are connected by nerves. They lie laterally beneath the œsophageal diverticulum and anterior portion of the mid-gut, and are not far removed from the salivary glands. Another small ganglion occurs above and in front of the proventriculus. (Plate 4, fig. 5.)

The Reproductive System.

The organs of the reproductive system are—

1. Ovaries.
2. Oviducts and common oviduct.
3. Mucus gland and duct.
4. Spermathecæ and ducts.

The ovaries occupy a variable position dependent upon the state of their development. In the newly-hatched mosquito they are small bodies lying in the fourth and fifth abdominal segments close by the posterior portion of the mid-gut, and attached to the body wall by numerous tracheæ. As they enlarge they push the mid-gut, hind-gut, and Malpighian tubes towards the ventrum, so that eventually the ovaries occupy nearly the whole of the posterior portion of the abdomen. Each ovary consists of very many follicular tubes, each containing egg follicles in different stages of development (see "Histology"). In the mature ovary the lower follicles have in every tube become the large completely-formed egg. (Plate 6, fig. 5.)

The oviducts are muscular tubes passing from the ovaries. They join beneath the rectum to form the common oviduct, which is still more abundantly supplied with muscle fibres, and which eventually opens beneath the anus.

The spermatheca is a chitinous sac, which in the impregnated female is filled with a mass of spermatozoa. Its duct is long and twisted and opens into the common oviduct near its termination.

The mucus gland, globular or ovoid in shape, opens by a short duct into the same region.

The Fat-body.—The adipose tissue is disposed in two ways.

1. As a general lining to the body wall, being nearly everywhere present directly beneath the cuticle (Plate 3, fig. 1), and

2. As lobular masses lying in among the organs and muscles. Thus a large pad lies over the compound thoracic ganglion, and sends processes which lie in among the salivary glands and other viscera. Other smaller masses lie in the head and abdomen. (Plate 1, fig. 3.)

Part II.—Histology.

Methods.—The examination of the fresh tissues frequently reveal structures not easily seen in fixed preparations. The tissues are best dissected out in normal saline of low tonicity, 0·3 or 0·4 per cent., as insect juices have a lower isotonic point than those of mammals. Better preparations of both tissues and included parasites are usually to be obtained by the use of fixed tissues. Several tissues (including the salivary glands and mid-gut) may, when dissected out, be spread by means of the edge of a slide or cover-glass, and rapidly dried. These, fixed and stained, give beautiful preparations of sporozoites, as well as certain parasites in the mid-gut, hind-gut, &c.

For fixing mosquitoes as a whole, watery solutions are not generally so good as alcohol, on account of the difficulty of penetration from the nature of the exoskeleton and the large amount of air contained in insect tissues: very good results are obtained by fixing and hardening in absolute alcohol, and proceeding at once to embed in paraffin. It is best, so soon as considerable hardening has taken place, to make a minute incision into both the thorax and abdomen. For fixing portions of or isolated organs of mosquitoes saturated solution of perchloride has advantages over alcohol and fixes the cells of the mid-gut extremely well. It does not penetrate, however, well into undissected mosquitoes. Picric acid gives good results with isolated organs. The changes in the mid-gut cells during digestion are well shown.

Both Culex and Anopheles, but especially the latter, cut readily in paraffin or celloidin. For staining smear preparations and sections hæmatein gives very good results: sporocysts and sporozoites, as well as the normal tissues, are well stained.

The stellate cells in connection with the tracheal endings upon the mid-gut, &c., are frequently well shown by gold chloride. Heidenhein's hæmatoxylin gives good results with the salivary glands, and also the muscle fibres in connection with the alimentary canal.

The Histology of the Alimentary Canal and Appendages.

The epithelial lining differs considerably in the mid-gut from either the fore-gut or hind-gut. In the mid-gut the possession of a marked

striated border by the epithelial cells is characteristic. The muscular fibres of the alimentary canal are striated throughout.

The Fore-gut.—The anterior portion of the fore-gut is lined by chitin and does not differ from the cuticle in structure. It consists of a single layer of cubical cells of small size. The œsophageal dilatation and its diverticula resemble one another in structure. In the adult mosquito they consist of an extremely delicate membrane formed of a single layer of flattened cells, with externally some scattered muscular fibres. In fresh preparations peculiar wrinklings of this membrane are seen which may appear like bundles of sporozoites. A similar appearance is seen in the dilated portion of the hind-gut just beyond the pylorus.

In the pupa the œsophageal diverticulum is seen passing backwards as a narrow tubular organ lying beneath the mid-gut. It is in this stage lined with well-marked cubical epithelium. In a freshly-hatched mosquito this organ is frequently undistended, and shows a narrow lumen surrounded by a single layer of large cells. These cells retain very little trace of protoplasm, which, however, may still be present in fine strands, and around the nucleus, which is pushed to the outer portion of the cell. (Plate 4, fig. 5.)

In the majority of mosquitoes the walls of the œsophageal diverticulum are crowded with micro-organisms and bodies which appear to be protozoal in nature.

The Mid-gut.—There is but little structural difference between the narrow anterior portion of the mid-gut which lies in the thorax and the posterior dilated portion which lies in the abdomen. In many insects there are cæcal tubes or pouches opening into the anterior portion of the mid-gut. These are, however, quite absent in the adult mosquito. The main thickness of the wall consists of epithelium; external to this is a thin coat of muscle fibres. (Plate 4, fig. 2.)

The epithelium consists of a single layer of large cells which are columnar in the undistended organ, but become flat and pavement-like when the organ is full of blood. They have a finely-reticulated protoplasm, which stains more deeply towards the free border. Stained with Heidenhein's hæmatoxylin, alcohol-hardened specimens are seen to contain numerous stained granules collected especially in the outer portion of the cell. These are especially abundant in the anterior portion of the mid-gut. They have also very frequently a number of small clear vacuoles (droplets) which become more frequent and of larger size towards the free border of the cell. The most marked feature of the cell is the clear striated border which is present in all the cells of the mid-gut, but absent in all other portions of the alimentary canal. The striated border is best marked in the undistended organ and becomes almost invisible in the fully-distended state when the cells are much flattened. (Plate 5, fig. 1.)

The nucleus of these cells is large and centrally situated. The chro-

matin is arranged in small stellate masses arranged circumferentially and centrally and connected with one another by fine threads of chromatin. There is a body which stains less deeply generally to be made out (karyosome) in the centre of the nucleus.

Occasionally young cells are to be seen near the basement membrane.

The muscular coat is very thin. It consists of an open mesh-work of long muscle fibres running longitudinally and circularly. In the large posterior portion of the mid-gut these fibres form a very regular series of large square or rhomboidal meshes. In the narrow anterior portion they are more closely approximated so that the muscular layer here is more evident in sections.

The individual muscle fibres are very long, fusiform, striated fibres. On the outer surface of the mid-gut lie numerous large branched cells in which the small tracheæ end, and from which bundles of minute structureless air tubes pass into the wall of the mid-gut. These cells are frequently well shown in gold chloride specimens. Similar cells occur throughout the viscera in connection with the tracheal endings. (See " Tracheal Endings.")

The Homologue of the Proventriculus.—Mention has been made in Part I of a fold occurring at the anterior extremity of the mid-gut. This consists of an invagination of a portion of the fore-gut into the mid-gut. The mid-gut is also folded in with the portion of fore-gut, so that in this region there is a double thickness of mid-gut wall as well as the fore-gut. There is an increase in the muscular fibres of the mid-gut at this point, especially the circular fibres, so that a very distinct mass is formed homologous to the proventriculus of many insects. There is no chitinous development, however, and the structure would appear to act only as a muscular sphincter. (Plate 1, fig. 3.)

The Hind-gut.—The nature of the epithelium and arrangement of the muscle fibres differs somewhat in different portions of the hind-gut. Structurally the small and large intestine are similar, whilst the dilatation beyond the pylorus, and especially the rectum, differ from these.

The dilatation which occurs at the origin of the Malpighian tubules is thin-walled and poorly supplied with muscle fibres. The cells lining it are small and flattened. (Plate 3, fig. 1.)

The intestine is lined with a single layer of large cubical cells; external to these is a muscular coat. The cells of the intestine have large nuclei which have a similar, though more open, arrangement of the chromatin than the nuclei of the mid-gut. The protoplasm is finely reticular, and stains less deeply than the cells of the mid-gut. Stained with Heidenhein's hæmatoxylin, no granules are present as in the cells of the mid-gut. They have no striated border. (Plate 4, fig. 3.)

In the rectum the cells become small and flattened. There are, however, here bodies usually termed rectal glands. These are papillæ

covered with a single layer of much hypertrophied cells resembling those lining the small intestine and colon. (Plate 4, fig. 4.)

The muscular system of the hind-gut is very similar to that of the mid-gut, consisting of very large fusiform, striated cells arranged circularly and longitudinally. The circular fibres in the small intestine lie outside the longitudinal, and pass spirally around the mid-gut. Towards the termination of the intestine longitudinal fibres also lie outside the circular. In the rectum and extending throughout the hind-gut and mid-gut, in both Anopheles and Culex, there are, in a large proportion of specimens, swarms of a flagellate organism. (Plate 5, fig. 3.)

The Salivary Glands.—The salivary acini lie in a cleft in the fat-body, which latter comes in close contact with the glands. Each gland acinus consists of a single layer of large cells limited externally by a delicate sheath (basement membrane) and internally by the intra-glandular duct wall. (Plate 5, figs. 6 and 7.)

In Anopheles the intra-glandular duct becomes larger as it approaches the termination of the acinus, and forms a large cavity.

In Culex the duct remains of the same diameter throughout the acinus, and terminates abruptly near the end of the acinus without any dilatation.

In both Culex and Anopheles there are two types of gland acinus. These are recognisable both in the fresh gland and in fixed specimens. From their appearance in the latter they may be termed

(1) The granular type.
(2) The clear or colloid-like type.

The Granular Type.—The greater portion of the acinus consists of cells whose nucleus and protoplasm has been pushed to the outer portion of the cell by a large mass of secretion which occupies almost the whole of the cell. In the fresh gland this secretion appears as a clear refractile substance, and can by pressure be made to exude from the cell in refractile globules. In specimens hardened in alcohol, this clear secretion appears as a granular mass occupying the greater portion of the cell. It stains faintly with hæmatein, and shows under high powers ($\frac{1}{16}$ oil immersion) a coarse reticulum and isolated globules, an appearance probably due to the precipitation or coagulation of the secretion by the alcohol. Considerable variations exist, however, in the appearance of this granular secretion both in the different mosquitoes and in different parts of the same gland. In anopheles the greater portion of the gland contains cells densely crowded with granular material. Very frequently, however, the terminal portion contains cells in which only a few large globular masses exist. (Plate 5, fig. 9.)

The protoplasm of the cell occupies in the fully-matured gland only

the extreme periphery, and the nucleus, which is much degenerated, is pushed to the outer portion of the cell, and usually lies in the angular interval left at the base of two or more contiguous cells. In the granular type of gland this disappearance of the protoplasm and nucleus from view is more pronounced than in the clear type of gland.

The Clear or Colloid-like Type.—Of the last-mentioned type there are two acini upon either side; of the present type there is but a single acinus upon either side, which usually lies between the two acini of granular type. (Plate 5, fig. 7.)

In the fresh gland the cell outlines are not so distinct as in the granular type, and the secretion when extended by pressure is much less refractive. In alcohol-hardened specimens, the acinar cells contain a large mass of clear homogeneous secretion which, as in the last-mentioned type, fills almost the entire cell, and pushes the protoplasm and nucleus to the periphery.

In the clear type, however, the protoplasm is always in greater amount than is the case with the granular type, and the nucleus never becomes so greatly degenerated. The clear homogeneous secretion stains readily with hæmatein, and may even stain quite deeply. With Heidenhein's hæmatoxylin it frequently becomes almost black. It resembles very much in appearance colloid substance as it is seen in the mammalian thyroid.

In Anopheles this substance also distends the central duct space within the acinus. In this situation an appearance is sometimes produced which resembles faintly-stained sporozoites, but which is a normal condition.

The Maturation of the Glands.—In freshly-hatched mosquitoes both types of acinus consist of large glandular cells arranged round the lumen. These contain a large centrally situated nucleus, and have protoplasm containing a large number of coarse granules staining with hæmatein. In the portion of the cell nearest the lumen a vacuole of varying size is situated. This is the commencement of the large mass of secretion which, in the mature gland, occupies the entire cell. In the granular type of acinus the vacuole contains granules; in the clear type it resembles the colloid-like secretion. (Plate 5, fig. 8.)

Further Variations in the Cells of the Salivary Acini.—In the granular type of gland the greater portion of the acinus is composed of cells of the character described above. A portion, however, usually exists which differs considerably in structure. This portion adjoins the duct, and may in Anopheles reach as much as one-quarter of the entire gland in length. In this portion of the gland the cells are much smaller than those containing the granular secretion, so that the diameter of the acinus is much less here, and a sudden increase takes place when the portion containing the granular secretion is reached. The cells lying towards the duct differ from those lying towards the acinar end of this

portion. There is, however, no line of demarcation between them, the one gradually becoming changed into the other. In the centre of each cell is a clear body, pushing the nucleus and protoplasm to the outer portion of the cell. Towards the duct end in the centre of this clear substance is a darker portion continuous with the duct lumen. As the cells come to lie nearer the distal portion, this central dark lumen becomes obliterated. This structure, though present in Anopheles, may be absent in Culex. In certain Culex another variation in the gland cells frequently occurs. The portion of the gland lying close to the duct, instead of being less in diameter is greater. The cells composing this portion are columnar in shape, with centrally situated nuclei and no contained secretion.

In certain specimens it is not uncommon to find cells occupying a peripheral position, and not approaching the lumen, which contain a substance resembling the colloid-like secretion of the clear type of gland.

Changes after Feeding.—Very little change occurs in the glands after feeding. They are for the most part still quite full of secretion. Probably a very small amount only of secretion is used with each puncture.

The Ducts.—The intra-acinar ducts vary in Culex and Anopheles. In Culex they remain narrow and tubular throughout the entire length of the gland. In Anopheles they become large spaces in both types of acini, but especially in the clear type. The duct is lined throughout by a clear homogeneous skeletal material which is continuous with a similar substance dividing the cells of the gland from one another. Into the duct the secretion-filled cell opens by means of a small opening.

The duct after leaving the acinus, consists of a thick-walled tube, with a central spiral thread resembling the spirals in the trachea. The wall is homogeneous, but contains many nuclei.

The Malpighian Tubules.—The Malpighian tubules are tubular bodies with cæcal ends, which open into the hind-gut. The cells are extremely large, being, next to the pericardial cells, the largest in the body. Each cell contains a large nucleus, and contains numerous large granules, which stain feebly with hæmatein, but powerfully with Heidenhein's hæmatoxylin. Numerous fatty granules are also present. Each cell is wrapped round a central lumen, the cells being arranged alternately, so that a zig-zag appearance is given in section. The inner portion of each cell is markedly striated, the lumen being thus bounded by a striated area. In relation with these tubules, a large number of tracheæ and tracheal end-cells exist.

In certain conditions the Malpighian tubule cells may be found quite free from granules, though otherwise unchanged. This change occurs in mosquitoes with large numbers of a flagellate organism (previously noted) in the rectum and hind-gut.

The Muscular System.—The muscular fibres of the mosquito are with-

out exception striated. Those of the wings differ in structure very much from those of the limbs and body segments. The muscle fibres of the alimentary canal are large fusiform cells, with a single large nucleus with some surrounding protoplasm. The muscle fibres in connection with the heart are much branched. (Plate 4, fig. 2.)

Many of the fibres contain a very marked sarcolemma and space between this latter and the fibre. This space is usually seen occupied by extremely delicate branching threads, which stain feebly with hæmatein.

In the pupæ there exist some large cells of peculiar nature in association with the sheaths of the muscle fibres.

The structure of insect muscle is described in many works on histology, and does not need repetition here.

The Tracheal System.—The larger tracheal vessels consist of a single layer of flattened cells with an inner chitinous layer. In smaller tubes the cells embrace the entire vessel, the nucleus frequently being bent around the lumen. The cells of the tracheal vessels contain numerous small clear vacuoles (chitin formation). The chitinous lining possesses a thickening in the form of a spiral thread, which may become unwound and lie stretched as a wavy thread in fresh preparations.

The smaller tubes contain the spiral thread until they become from 2 to 5 μ in diameter. They then divide to form bundles of excessively minute air capillaries, which enter among the tissue cells. The division into capillaries takes place in the substance of large branched cells situated at the termination of the tracheal vessels. The cells often appear cribriform in section from the number of air capillaries. These cribriform cells in connection with the tracheal endings are well seen in the mid-gut and Malpighian tubules. They are, however, seen best of all in the undeveloped ovary of the newly-hatched mosquito, which is extremely rich in bundles of capillary air tubes.

The Vascular System.—The dorsal vessel is a delicate walled tube composed of longitudinal and oblique fibres with a nucleated inner layer. The fibres may be traced directly from the terminations of the branched alary muscle fibres. The alary fibres break up into fibres which pass in close connection with the large pericardial cells, and eventually form (1) fibres passing into the dorsal vessel as longitudinal fibres, (2) fibres joining in an anastomosis in connection with the floor of the dorsal vessel. (Plate 4, fig. 1.)

The pericardial cells are extremely large cells lying on either side of the dorsal vessel throughout its whole extent. They are by far the largest cells in the mosquito, varying from 30 μ to 50 μ in long. diameter. They are elongate or pear shape in form and contain several nuclei. The nuclei usually show signs of degeneration. The peripheral portion of the cell stains more deeply than the central portion, which contains the nuclei and small stained granules. There are a considerable

number of masses of a light yellowish pigment resembling that found in the large visceral ganglia cells. The fibres from the branches of the alary muscles pass over and around the pericardial cells to reach the dorsal vessel. From their structure and situation the pericardial cells appear to be of the nature of ganglion cells. (Plate 5, fig. 5.)

The Fat-body.—The fat-body, both where it occurs as a portion of the body wall and where it lies as free lobulated masses, consists of cells containing numerous oil globules. The cells are of considerable size, and their borders may be frequently traced as polygonal areas. The nuclei are oval in shape with a central mass of chromatin and chromatin threads. Besides oil globules the cells contain granules staining with hæmatein, and minute droplets of a highly refractile, dark substance, which gives the appearance of pigment. These droplets are larger in amount in old mosquitoes than in those freshly hatched.

The Nervous System.—The ganglia of the ganglionic system consist of an outer portion of nerve cells and an inner portion of non-medullated nerve fibres. Considerable complexity exists in the larger ganglia, especially the head ganglia. (Plate 5, fig. 4.)

The ganglia of the visceral system differ greatly from those of the ganglionic system. The ganglion cells are few in number and of large size. They possess clear reticular protoplasm, a little denser around the periphery than in the centre. Around the inner margin of the denser peripheral portion small stained points are arranged. In the centre a variable number of granules of yellowish pigment exist. (Plate 6, fig. 1.)

The Reproductive System.—Each ovary consists of a large number of follicular tubes whose lower ends open into the ovarian tube, and whose upper ends terminate in a delicate supportiug filament (terminal filament). The apex of the ovary is formed of a single follicular tube whose filament is attached to the fat-body of the 4th segment.

Around the whole ovary there is a delicate nucleated sheath.

Each follicular tube contains one or more egg-follicles in different stages of development. In the freshly-hatched mosquito each follicular tube contains an undeveloped egg-follicle. As this develops, a second and a third undeveloped follicle appear above it, which again undergo development into mature eggs. The follicle at first consists of two to four large cells with large nuclei surrounded by a single layer of smaller epithelial cells. (Plate 6, figs. 2, 3, 4.)

The central cells then increase in size and number, so that many very large cells are contained in the now enlarged follicle. The surrounding epithelial cells also become larger, and rapidly increase in number so as to form a layer of regular cubical cells surrounding the follicle. The central cell nearest the ovarian tube is the ovum, the rest are nurse cells, and eventually disappear. Both the ovum and the nurse cells increase greatly in size. The nurse cells have clear

protoplasm and extremely large nuclei, which exhibit karyokinetic figures. The ovum contains very numerous yolk granules, which occupy the whole of its substance, except a thin coating of granular protoplasm. Still later this thin external layer can only with difficulty be made out. (Plate 6, fig. 4.)

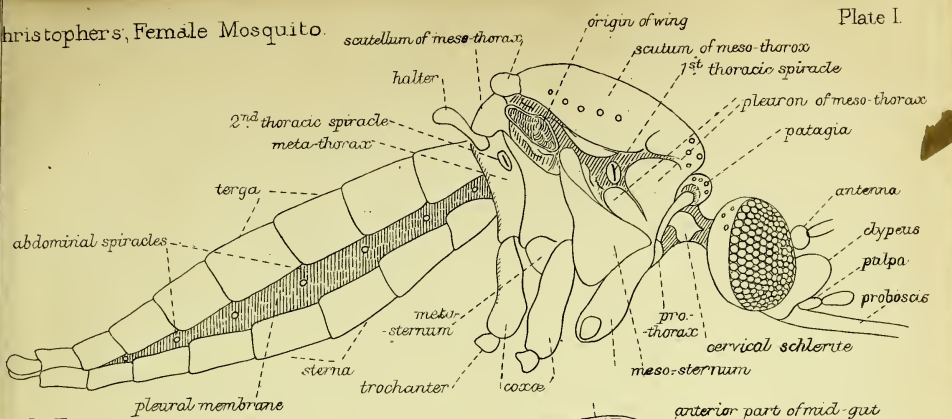
The nucleus of the ovum undergoes very pronounced changes. It appears as an irregular mass, staining uniformly with nuclear stains. This mass becomes more and more distorted and broken up, and eventually disappears. It may frequently, however, be seen as irregular masses of staining material even in the mature egg. A portion of the nucleus is seen very early to be separated off from the rest, often surrounded by the latter. This portion (female pronucleus) is small and difficult to detect in sections in the more mature ovum. As the ovum increases still more rapidly in bulk, the nurse cells become crowded into the distal portion of the follicle and eventually disappear, so that, in the mature egg, no trace of them is to be seen. The epithelial layer surrounding the follicle becomes much flattened, and forms eventually a covering to the egg (chorion). The outer portion of this covering (exochorion) is transparent, and marked with oblique parallel markings. Over the proximal end, *i.e.*, the end lying towards the ovarian tube, the chorion forms a globular mass ornamented with rows of pits. This is the micropylar apparatus through which the spermatozoa penetrate the ovum.

Frequently in Anopheles a large portion or the whole of the adult ovum consists of a mass of sporozoa. These consist of numerous small cysts, each containing eight round or crescent-shaped bodies, each with a central chromatin spot. (Plate 6, figs. 6, 7.)

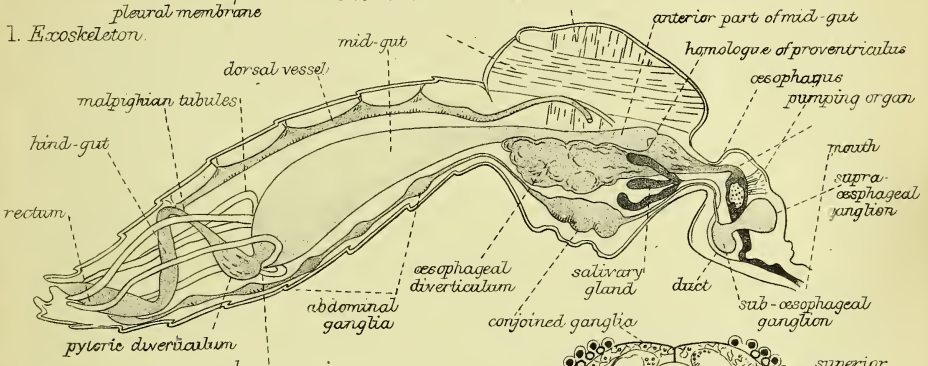
The ovarian tube arises in the centre of the ovary, and receives on all sides the follicular tubes. It is lined with a single layer of small cubical epithelium. After passing out of the ovary, a considerable number of striated muscular fibres are arranged in a loose network around it, and pass from it to surrounding structures. There are also muscular fibres in the ovary itself in connection with the ovarian tube and egg-follicles.

The spermatheca consists of a chitinous sac, with large cells lying externally. These resemble the cells of the cuticle, and contain droplets. They do not cover the whole of the surface of the spermatheca. The contents of the spermatheca in the fertilised insect consist of a mass of spermatozoa, which, in the fresh state, may be seen revolving with great rapidity within the sac. The spermatozoa have a narrow, slightly-curved head and a long tail. The duct of the spermatheca is narrow and thick-walled, and contains muscular fibres. Certain large cells lie in connection with the duct externally. The mucus gland contains cells filled with secretion. There are small nuclei in connection with the intra-acinar duct. (Plate 6, figs. 8, 9.)

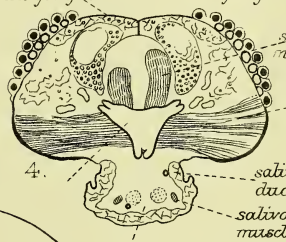
hristopher's, Female Mosquito.

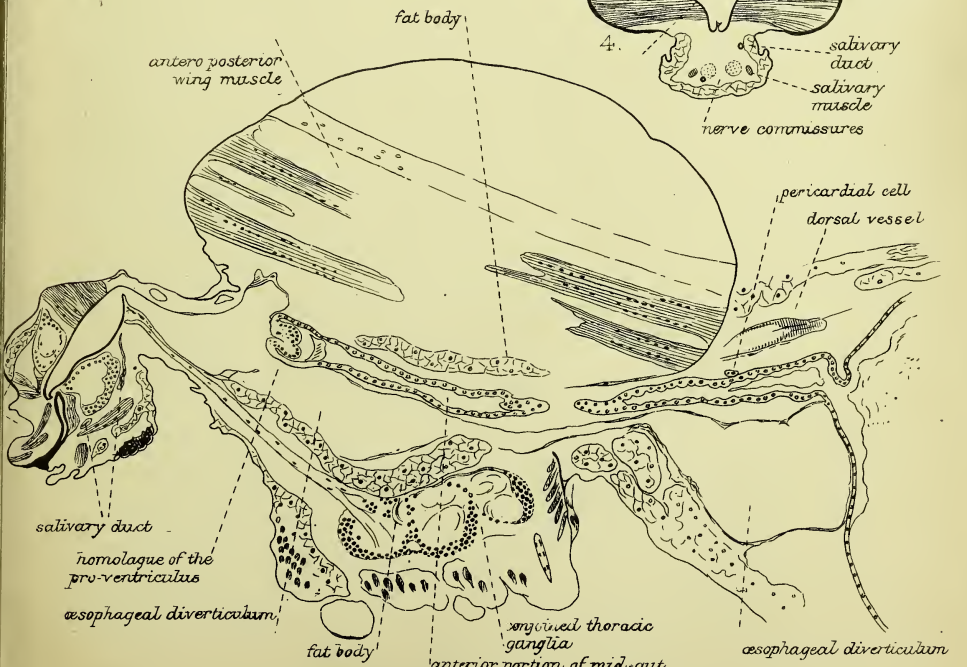


1. *Exoskeleton.*



2. *General view of organs.*



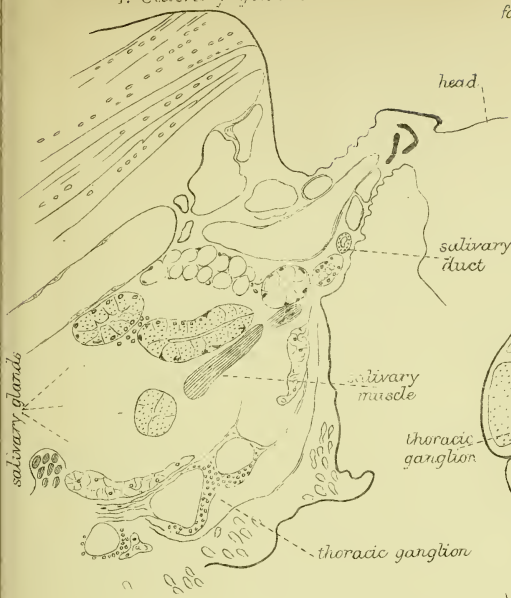


3. *Median longitudinal section through thorax.*

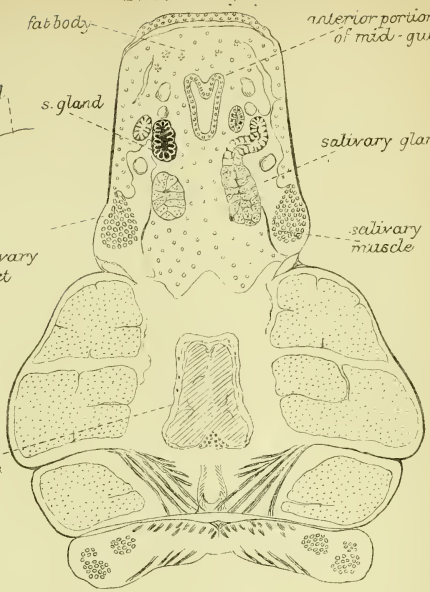
West, Newman lith.

ristophers, Female Mosquito.

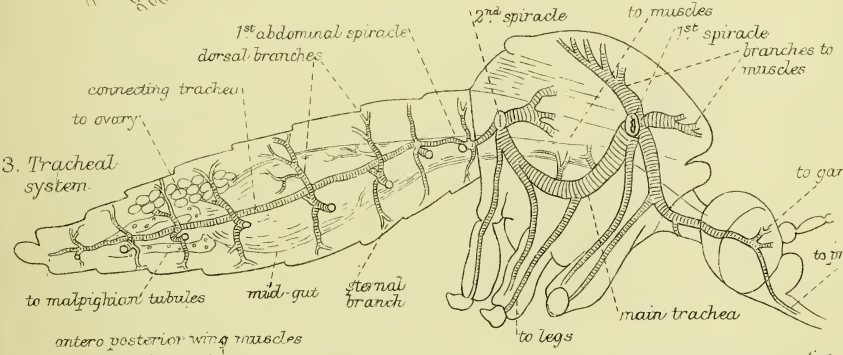
1. Salivary glands.

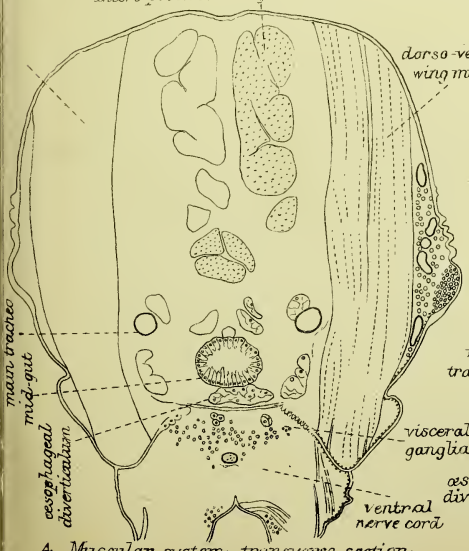


2. Salivary glands.

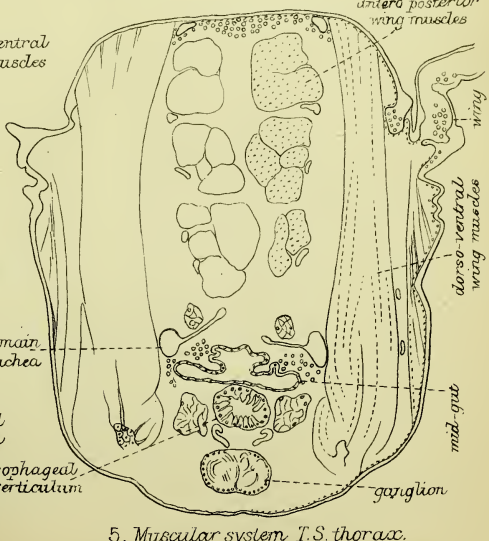


3. Tracheal system.



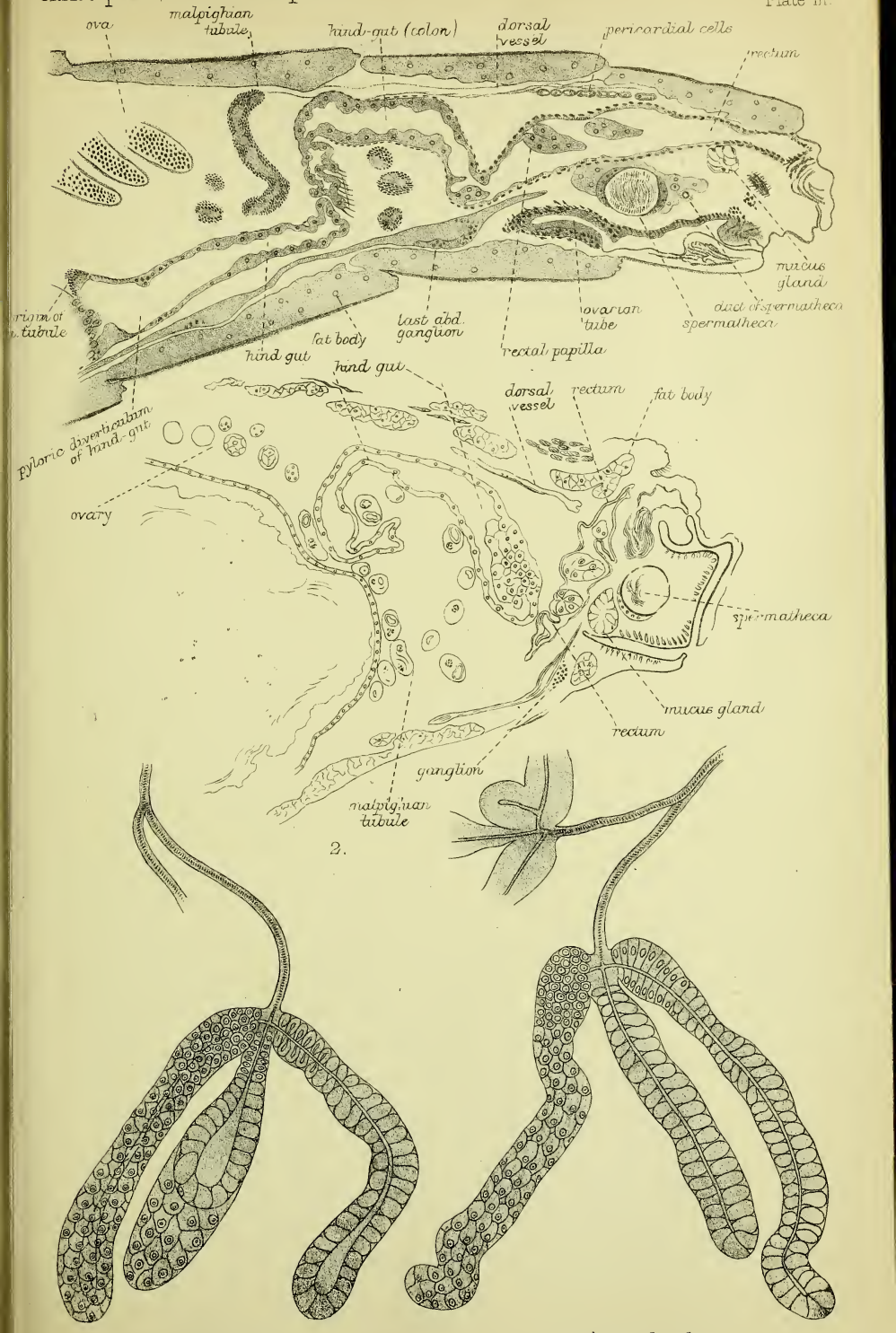


4. Muscular system transverse section of thorax.



5. Muscular system T.S. thorax.

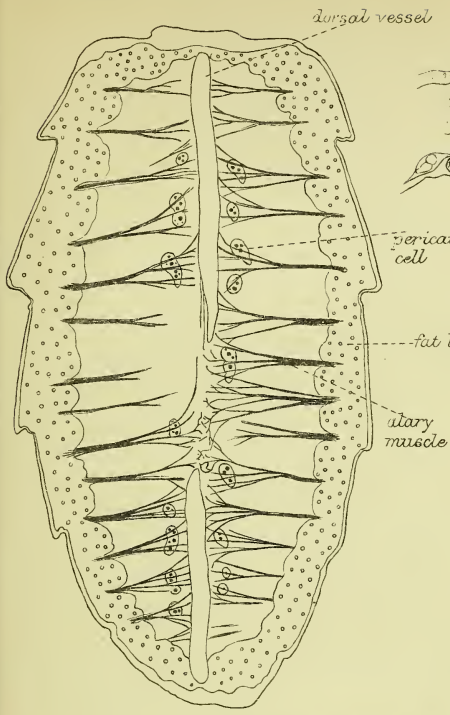
West, Newman lith.



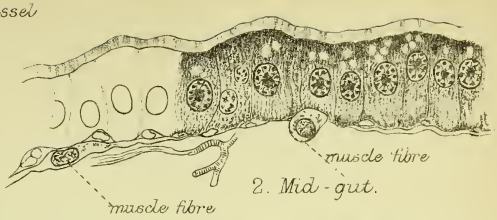
3. *Salivary glands*
Anopheles.

4. *Salivary glands*
Culex.

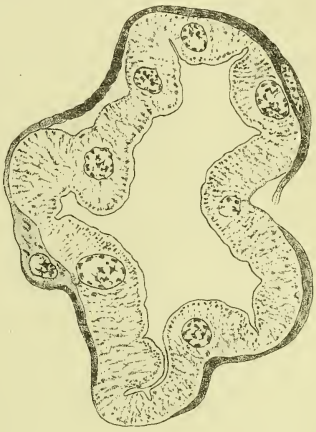
West, Newman lith.



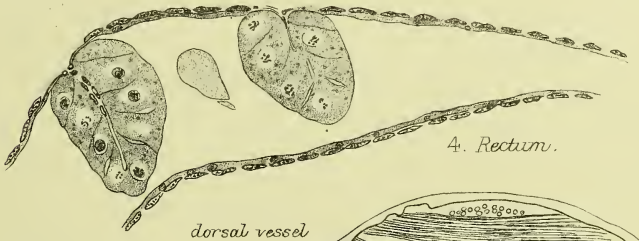
1. Dorsal vessel.



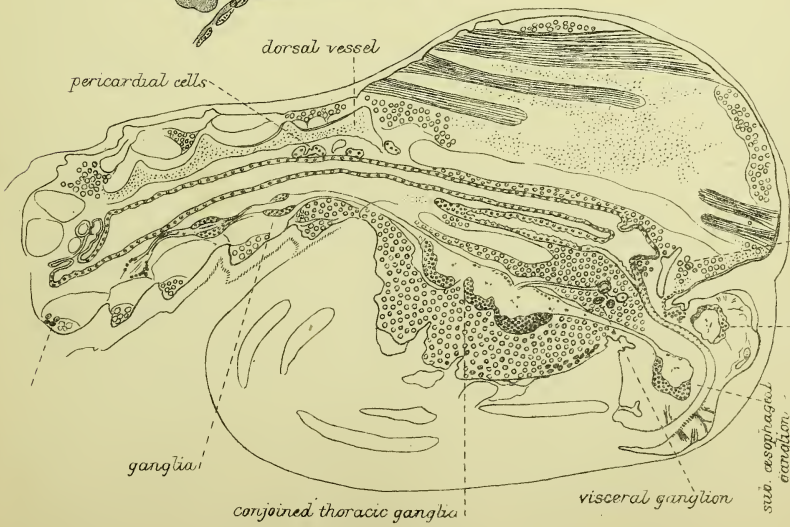
2. Mid-gut.



3. Hind-gut.



4. Rectum.

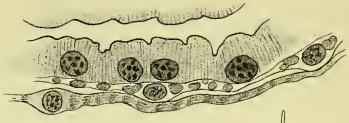


5. Nervous system. L.S. Section of nympha.

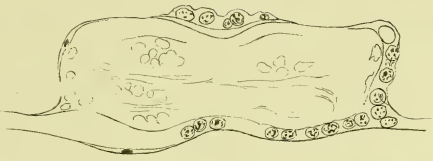
West, Newman lith.

1. *Mid-gut distended*

2. *Hind-gut*

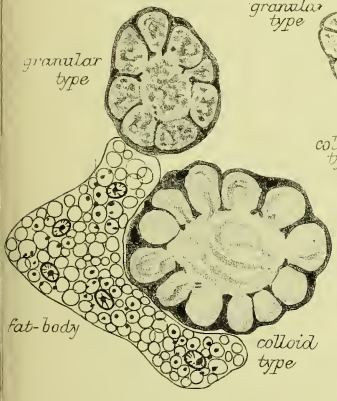


3. *Parasites in hind-gut.*

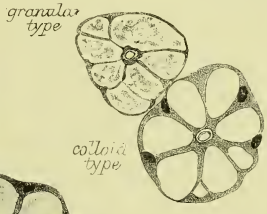


4. *Ganglion*

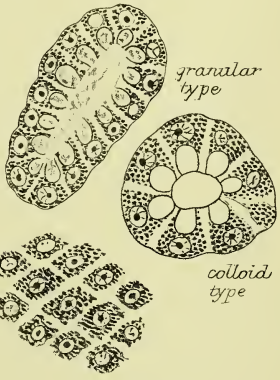
5. *Pericardial cell and alary muscle.*



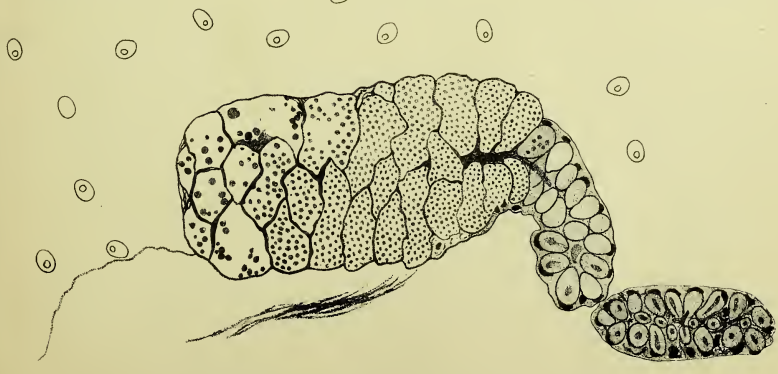
6. *Salivary glands anopheles.*



7. *Salivary glands culex.*

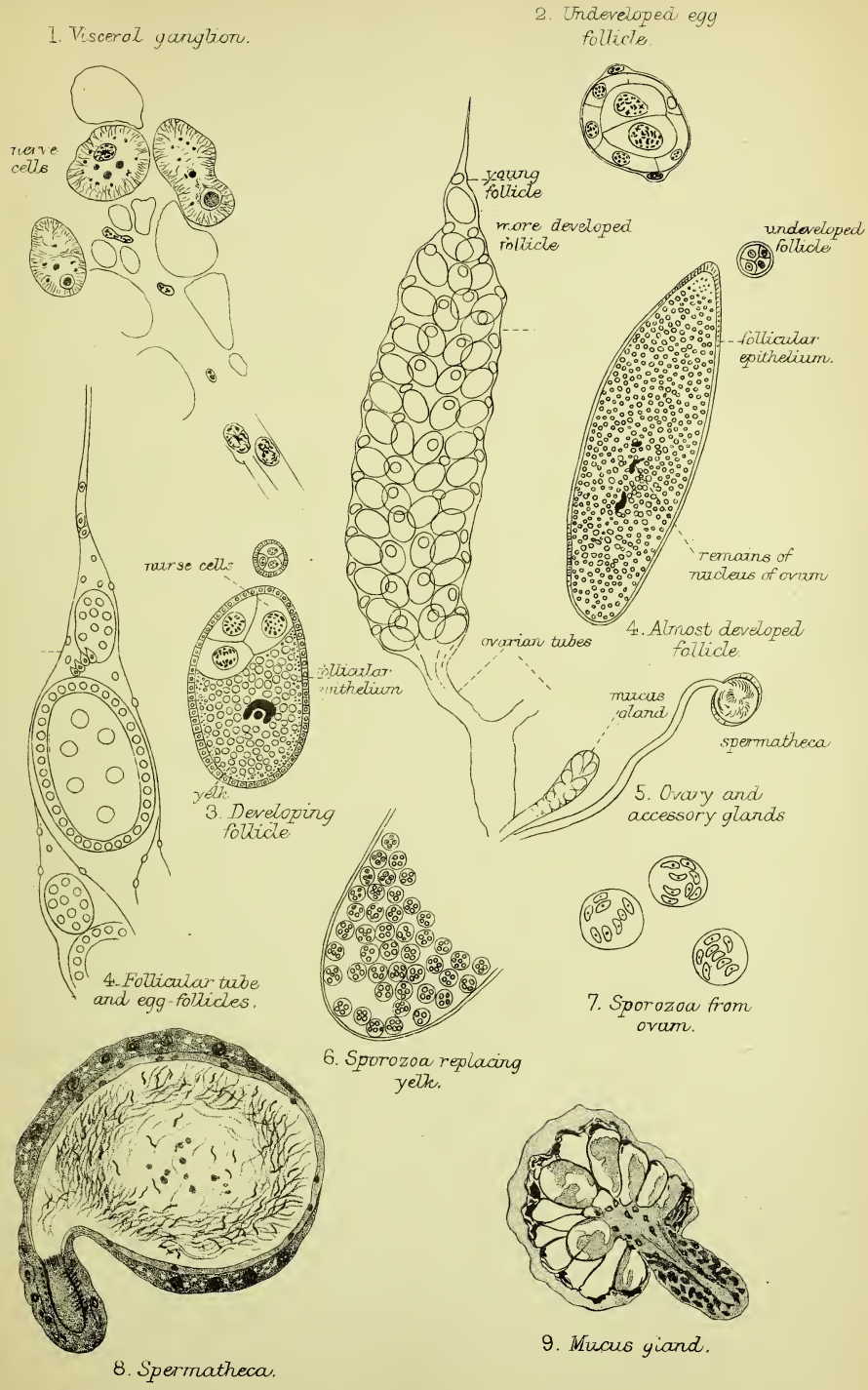


8. *Glands of newly hatched anopheles.*



9. *Longitudinal section of salivary gland of anopheles, granular type.*

West, Newman lith



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